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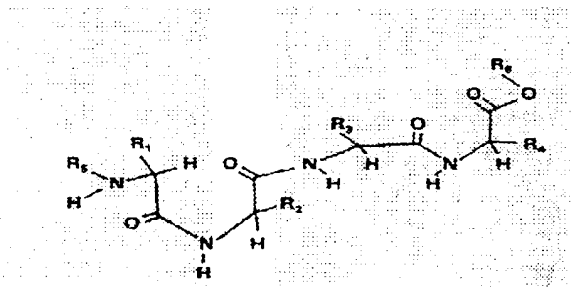
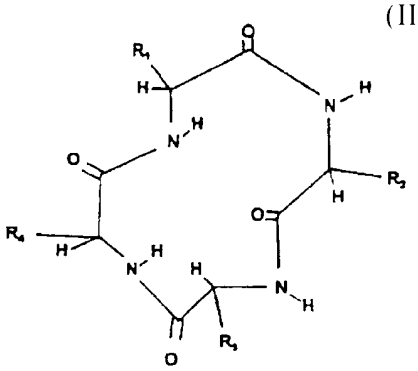
(54) PEPTIDES UTILISES COMME ANALGESIQUES

(54) PEPTIDES AS ANALGESICS

(I)



(II)



(57) Il n'existe pas de remede efficace pour traiter la douleur chronique résultant de l'inflammation et de la neuropathie. L'histogranine (HN), le peptide de la médullo-surrénale, et son analogue chimiquement stable, [Ser[1]]HN, soulagent le même type de douleur que celle bloquée par les implants de médullo-surrenale dans la moelle épinière. Toutefois, l'administration de ces peptides comporte plus d'avantages que la greffe de tissus de la médullo-surrenale ou de cellules chromaffines dans la moelle epinière. En outre, il est possible de modifier la structure des peptides HN pour améliorer leur efficacité et leur durée d'action tout en évitant les effets secondaires des analgésiques opioïdes, des dérivés de l'aspirine et des anti-inflammatoires non stéroïdiens. Le fragment HN-(7-10), Gly-Gln-Gly-Arg (SL-99), est un analgésique aussi puissant que le peptide parent HN dans l'essai de l'induction de la douleur chez la souris. Ses analogues, [Ala[9]]HN-(7-10) (SL-100 : Gly-Gln-Ala-Arg) et [Arg[7], Ala[9]]HN-(7-10) (SL-101 : Arg-Gln-Ala-Arg), cyclo-(SL-100) (ou SL-102) et le peptide parent [Ser[1], Ala[9]]HN (SL-104 : Met-Asn-Tyr-

(57) No effective remedy exists for the treatment of chronic pain resulting from inflammation and neuropathy. The adrenal medullary peptide, histogranin (HN), and its chemically stable analogue, [Ser[1]]HN, alleviate the same type of pain as that blocked by spinal adrenal medullary implants. However, the administration of these peptides is more advantageous than the transplantation of adrenal medullary tissues or chromaffin cells into the spinal cord. In addition, the structure of HN peptides can be modified to improve their efficacy and length of action while avoiding the side-effects of opioid analgesics, aspirin derivatives and non-steroidal antiinflammatory agents. The HN fragment-(7-10), Gly-Gln-Gly-Arg (SL-99), is an analgesic as potent as the parent peptide HN in the mouse writhing pain assay. Its analogues, [Ala[9]]HN-(7-10) (SL-100: Gly-Gln-Ala-Arg) and [Arg[7], Ala[9]]HN-(7-10) (SL-101: Arg-Gln-Ala-Arg), cyclo-(SL-100) (or SL-102) and the parent peptide [Ser[1], Ala[9]]HN (SL-104: Met-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Ala-Arg-Thr-Leu-Tyr-Gly-Phe), show improved potency as

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Ala-Leu-Lys-Gly-Gln-Ala-Arg-Thr-Leu-Tyr-Gly-Phe), démontrent une plus grande puissance que HN. Les formes linéaire et cyclique de SL-100 et de SL-101 sont présentées avec les formules générales suivantes : formule I (linéaire) formule II (cyclique) (voir formules I et II), dans lesquelles R_1, R_2, R_3, R_4, R_5 et R_6 représentent H (SL-100) ou $(CH_2)_2$-NH_2 (SL-101), $(CH_2)_3$-NH-C(=NH)-NH_2, CH_3, $(CH_2)_2$-NH_2, H et H, respectivement, ou des substituants apparentés. Les sels, les esters et les amides utilisables en pharmacie compris dans les composés des formules I et II, qui sont utiles pour induire l'analgésie chez les animaux, ainsi qu'une méthode pour induire l'analgésie chez un animal en proie à des douleurs, comprenant l'administration d'une quantité thérapeutiquement efficace des composés des formules I ou II à l'animal sont également dévoilés.

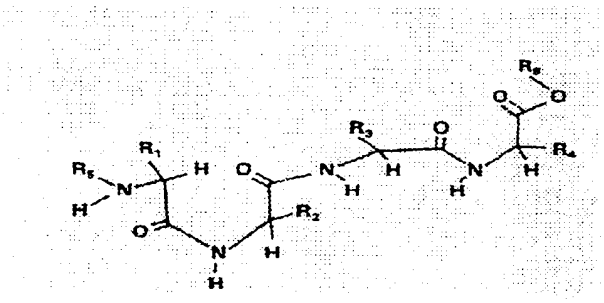
compared with HN. The linear and cyclic forms of SL-100 and SL-101 are presented with the following general Formulae: Formula I (linear) Formula II (cyclic) (see formulae I and II) wherein R_1, R_2, R_3, R_4, R_5 and R_6 are H (SL-100) or $(CH_2)_2$-NH_2 (SL-101), $(CH_2)_3$-NH-C(=NH)-NH_2, CH_3, $(CH_2)_2$-NH_2, H and H, respectively, or related substituents. The pharmaceutically-acceptable salts, esters and amides comprised in formulae I and II compounds, which are useful for inducing analgesia in animals, and a method for inducing analgesia in an animal in need thereof comprising administering a therapeutically-effective amount of Formula I or Formula II compounds to the animal.

LINEAR AND CYCLIC GLY-GLN-ALA-ARG, ARG-GLN-ALA-ARG AND RELATED TETRAPEPTIDES AS POTENT ANTINOCICEPTIVE AGENTS

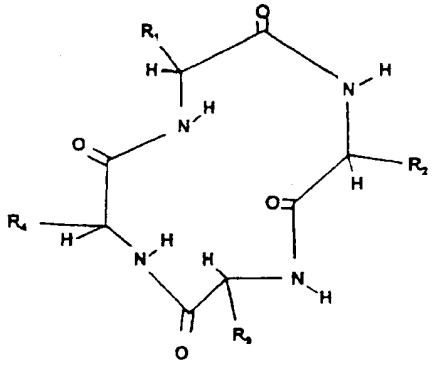
ABSTRACT

No effective remedy exists for the treatment of chronic pain resulting from inflammation and neuropathy. The adrenal medullary peptide, histogranin (HN), and its chemically stable analogue, [Ser^1]HN, alleviate the same type of pain as that blocked by spinal adrenal medullary implants. However, the administration of these peptides is more advantageous than the transplantation of adrenal medullary tissues or chromaffin cells into the spinal cord. In addition, the structure of HN peptides can be modified to improve their efficacy and length of action while avoiding the side-effects of opioid analgesics, aspirin derivatives and non-steroidal antiinflammatory agents. The HN fragment-(7-10), Gly-Gln-Gly-Arg (SL-99), is an analgesic as potent as the parent peptide HN in the mouse writhing pain assay. Its analogues, [Ala^9]HN-(7-10) (SL-100: Gly-Gln-Ala-Arg) and [Arg^7, Ala^9]HN-(7-10) (SL-101: Arg-Gln-Ala-Arg), cyclo-(SL-100) (or SL-102) and the parent peptide [Ser^1, Ala^9]HN (SL-104: Met-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Ala-Arg-Thr-Leu-Tyr-Gly-Phe), show improved potency as compared with HN. The linear and cyclic forms of SL-100 and SL-101 are presented with the following general Formulae:

Formula I (linear)



Formula II (cyclic)



wherein R_1, R_2, R_3, R_4, R_5 and R_6 are H (SL-100) or $(CH_2)_2$-NH_2 (SL-101), $(CH_2)_3$-NH-C(=NH)-NH_2, CH_3, $(CH_2)_2$-NH_2, H and H, respectively, or related substituents. The pharmaceutically-acceptable salts, esters and amides comprised in formulae I and II compounds, which are useful for inducing analgesia in animals, and a method for inducing analgesia in an animal in need thereof comprising administering a therapeutically-effective amount of Formula I or Formula II compounds to the animal.

LINEAR AND CYCLIC GLY-GLN-ALA-ARG, ARG-GLN-ALA-ARG AND RELATED TETRAPEPTIDES AS POTENT ANTINOCICEPTIVE AGENTS

BACKGROUND OF THE INVENTION

Histogranin (HN: H-Met-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-COOH) was first coined by our laboratory as an adrenal medullary peptide possessing N-methyl-D-aspartate (NMDA) receptor antagonist activity as assessed by its ability to block NMDA-induced convulsions in mice (1) and to produce phencyclidine(PCP)-like behaviourial effects in rats (2). The radiolabelled peptide possesses a specific receptor on rat brain membranes (3). Binding of HN to its receptor was demonstrated to affect the activity of specific modulators (Gly, dextromethorphan) of the NMDA receptor (4,5).Herein, two tetrapeptides [Gly-Gln-Ala-Arg (SL-100) and Arg-Gln-Ala-Arg (SL-101)] derived from the structure of the minimal active core peptide, HN-(7-10) (SL-99), and their cyclic forms [cyclo-(-Gly-Gln-Ala-Arg-): SL-102] and [cyclo-(-Arg-Gln-Ala-Arg-): SL-103] and analogues are proposed as pain relieving agents.

It has been known for a long time that neuropathic pain, eg. pain induced by peripheral nerve injury as a result of a chronic disease or some inflammatory processes, is manifested by hyperalgesia (exaggerated nociceptive responses to noxious stimulation), allodynia (nociceptive response to innocuous stimulation) and spontaneous pain. Compelling evidence indicates that activation of spinal cord NMDA

receptors contributes to the hyperalgesia that occurs following peripheral nerve injury or inflammation. Thus, administration of either competitive (AP-5) or non-competitive (MK-801) NMDA receptor antagonists powerfully reduces thermal hyperalgesia in animal models of neuropathy (6), carrageenan-induced acute peripheral inflammation (7), heat-injury (8) and formalin-induced pain (9). Likewise, activation of NMDA receptors within the spinal cord has been shown to play a role in the development of tolerance to the analgesic effects of morphine (10). In this regard, various studies indicate that agents antagonizing the NMDA receptor can prevent morphine tolerance (11-13). However, currently used NMDA receptor antagonists produce major side-effects, including motor dysfunction, learning impairment, hallucinations etc...

In 1990, Sagan and colleagues (14) have devised an experimental model for the alleviation of chronic pain in which the hyperalgesic state caused by sciatic nerve injury in rats was completely blocked by adrenal medullary implants into the spinal cord. The beneficial effects of the transplantation of adrenal chromaffin cells into the spinal subarachnoid space were also observed in rats models of arthritis (15) and depression (16). In addition, the analgesic effects of adrenal medullary transplants did not display tolerance and morphine cross-tolerance upon intermittent administration of nicotine (which evoked the release of the analgesic factor(s) from the adrenal medullary implant) (17) and they were accompanied by a reduction of spinal nerve degeneration (18). At that time, most analgesic and neuroprotective effects of adrenal

medullary implants were thought to be produced by opioid peptides and catecholamines released from transplanted adrenal chromaffin cells (19), although such effects were not completely blocked by naloxone (an opioid antagonist) and phentolamine (an adrenergic antagonist), alone or in combination.

Recently, Dr. Sagan and colleagues reported that the analgesic effects of the adrenal implants in rat models of chronic pain and inflammation are mimicked by the peptide [Ser^1]HN (20, 21, 22). In their experimental protocols, a relatively low dose of [Ser^1]HN (1 nmol, i.t.) was shown to block chronic pain induced by peripheral neuropathy, formalin-induced pain and direct application of NMDA. In the formalin test, HN produced analgesia in the late (NMDA-dependent) phase, but not early (NMDA-independent) phase of the pain assay (21).

We have also observed that HN and related peptides are potent analgesics in the mouse writhing test (23). In this acetic acid-induced pain assay, central (i.c.v., 0.5-50 nmol) and peripheral (i.p., 5 μmol/ kg) administrations of HN and related peptides produced strong (up to 100%) analgesia with no motor side-effect. The analgesic effects of [Ser^1]HN (50 nmol, i.c.v.) were blocked by the NMDA receptor antagonists, CPP and MK-801, but not by the opiate antagonist, naloxone, suggesting that the analgesic properties of HN and related peptides involve NMDA receptor-mediated mechanisms (23). Thus, adrenal medullary HN may be one of the factor(s) that mediate the non-opioid antinociceptive effects of spinal

adrenal medullary implants; the adrenal peptide may also be the mediator of some physiological phenomena such as stress-induced analgesia, a physiological condition that is known to involve NMDA receptor mechanisms (24).

HN and related peptides at analgesic doses in mice do not display any noticeable behaviourial activity (in the rotarod assay and by gross observation). However, these peptides display marked analgesia after both central (i.c.v.) or peripheral (i.p.) administration (23). It is presumed that the analgesic effects of HN result from its interaction with both central and peripheral receptors. Interestingly, HN was shown to bind to specific receptors located in the brain (3) and on peripheral blood lymphocytes (25). Analgesia may also result from the blockade by HN of the formation of pain mediators such as prostaglandins. Preliminary results indicate that HN blocks the synthesis of prostaglandin-E2 in isolated rat alveolar macrophages in response to lipopolysaccharide (unpublished observations). The mechanism by which HN and related peptides produce analgesia is still unknown, but the possible involvement of the dextromethorphan binding site on the NMDA receptor complex is suggested by the close correlation that exists between the ability of HN and related peptides to produce analgesia and potentiate the binding of [^{3}H]dextromethorphan, a non-competitive NMDA antagonist, to rat brain membranes (4).

Chronic pain may result from multiple causes including pain related to inflammation, peripheral nerve injury, cancer, AIDS, diabetes etc... The drugs that are

being used for the treatment of chronic pain (derivatives of aspirin and non-steroidal antiinflammatory agents) have very limited efficacy and they produce important side-effects. They interfere with blood coagulation, they cause and/or exacerbate peptic ulcer etc... NMDA receptor antagonists are effective in animal models of neuropathy, but these latter compounds produce behaviourial side-effects (motor impairment, learning impairment, locomotion, ataxia etc...) that hamper their use as therapeutic agents. Morphine and opioid analgesics show no or few beneficial effects: they produce marked tolerance, addiction and withdrawal syndromes and they are not effective against neuropathic pain. Based on the data observed with the adrenal implants, it is expected that the adrenal medullary peptide, HN, and its analogues will not only be effective as analgesic agents, but they may also display neuroprotective activity and alleviate tolerance to morphine.

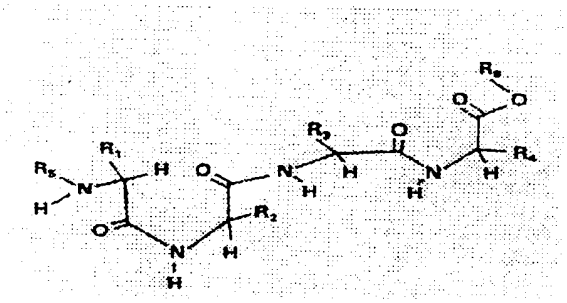
OBJECTS OF THE INVENTION

An object of the invention is to provide a peptide, for example HN-(7-10): Gly-Gln-Gly-Arg (SL-99), as minimal core peptide comprising amino acids of only the L-configuration that produces analgesia.

Another object of the invention is to provide two analogues of HN-(7-10): [Ala^9]HN-(7-10): Gly-Gln-Ala-Arg (SL-100) and [Arg^7, Ala^9]HN-(7-10): Arg-Gln-Ala-Arg (SL-101), with improved analgesic potencies.

Another object of the invention is to provide a series of analogues of SL-100 and SL-101 with the following general Formula I:

Formula I



and the pharmaceutically-acceptable salts, esters and amides, wherein:

R_1 is hydrogen, an alkyl or a basic radical (the term alkyl as used herein means a hydrocarbon radical having from one to ten carbon atoms, which can be a straight or branched chain, and including from zero to four carbon-carbon double or triple bonds. Representative of such radicals are methyl, ethyl, n-propyl, isopropyl, n-butyl, 2-ethyl-hexyl and the like. The term basic as used herein means $(CH_2)_n$-NH_2 or $(CH_2)_n$-NH-C(=NH)NH_2, "n" each independently an integer from 0 to 10);

R_2 is an amide radical such as $(CH_2)_n$-$CONH_2$ "n" an integer from 0 to 10;

R_3 is hydrogen or an alkyl radical as defined above;

R_4 is a basic radical as defined above;

R_5 is hydrogen or an acetyl or an alkyl radical as defined above;

R_6 is hydrogen, alkyl, alkyl carbonyl, alkoxy carbonyl, amino carbonyl, alkylaminocarbonyl, dialkylamino, carbonyl, $(CH_2)_n$-benzyl, $(CH_2)_n$-phenyl ("n" an integer

from 1 to 10).

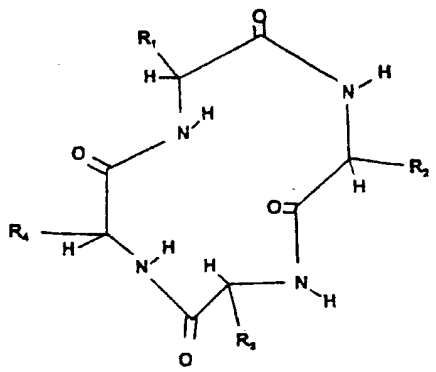
$O\text{-}R_6$ is replaced by R_7 (not shown), R_7 being amino, hydroxy, alkoxy, alkylamino, dialkylamino, or alkoxyaryl.

$O\text{-}R_6$ is replaced by R_7, R_7 being independently positions 11 to 15 in HN and represented by Thr^{11}-Leu-Tyr-Gly-Phe^{15}, Thr^{11}-Leu-Tyr-Gly^{14}, Thr^{11}-Leu-Tyr^{13}, Thr^{11}-Leu^{12} and Thr^{11}, or homologous peptides or amino acids, for example, Thr^{11} may be exchanged for Ser, Leu^{12} may be exchanged for Ala, Val or Ile, Tyr^{13} may be exchanged by Phe or diiodotyrosine, Gly^{14} may be exchanged for Ala, Val, Leu or Ile, and Phe^{15} may be exchanged for Tyr or diidotyrosine.

Another object of the invention is to provide the structure of the cyclic tetrapeptides, cyclo(-Gly-Gln-Ala-Arg-) (SL-102) and cyclo(-Arg-Gln-Ala-Arg-) (SL-103), as potent and long-lasting analgesic agents.

Another object of the invention is to provide a series of analogues of SL-102 and SL-103, with the following general Formula II:

Formula II



wherein R_1, R_2, R_3 and R_4 are defined as above.

In the peptides of general formulae I and II, the chiral carbons of the peptide backbone may each independently be of either the D- or L-configuration. It is preferred that they are of the L-configuration.

Another object of the invention is to provide the structure of an analogue of the pentadecapeptide [Ser^1, Ala^9]HN (SL-104), as a potent analgesic agent.

The invention further extends to fragments of histogranin of greater than 4 residues, as well as homologues of histogranin and homologues of the fragments. By homologue is intended a peptide in which the sequence differs from that of the parent by replacement of 1 to 4 amino acid residues with other amino acids.

The cyclic peptides of formula II are cyclized in a head-to-tail fashion.

Peptides of the general formula I and II are prepared using techniques of peptide chemistry. They may be prepared in solution or by solid-phase methods. Examples of preferred synthetic methods are as follows:

Peptides of Formula I may be synthesized for example, as described by Prasad et al. (Can. J. Physiol. Pharmacol. 73: 209-124, 1995) by the use of preformed symmetrical anhydrides (Lemaire et al., J. Med. Chem. 21: 1232-1235, 1978; with the exception of Boc-Arg, Boc-Asn and Boc-Gln) of Boc-amino acids (Bachem California) with a solid-phase method (Merrifield, J. Am. Chem. Soc., 85: 2149-2154, 1963) on chloromethylated polystyrene-divinylbenzene resin (benzhydrylamine or oxime

resins can also be used to generate the various C-terminal substituted groups of Formula I according to Bodanszky and du Vigneaud (J. Am. Chem. Soc. 81: 5688, 1958). The various steps of the automatic coupling cycles are described by St. Pierre, Gaudreau, Drouin, Regoli and Lemaire (Can. J. Biochem. 57: 1084-1089, 1979). Boc-Arg, Boc-Asn and Boc-Gln are coupled to the deprotected N-terminal group of the growing peptide-resin by the method of Coste et al. (Tetrahedron Lett. 31: 205-208, 1990). Side-chain protecting groups are as follows: Arg, Tos; Lys, 2-Cl-Z; Thr and Ser, Bz; Tyr, 2,6-dichloro-Bz; His, Boc; Asp and Glu, Obzl. The completed peptides are cleaved from the resin and deprotected with liquid hydrogen fluoride (HF) and purified by successive chromatographies on Sephadex G-10 and high performance liquid chromatography (HPLC) on Bio-Sil C18 column (Waters, Milford, MA). The purity and identity of synthetic peptides is verified by analytical HPLC on μ-Bondapak C18 column (Waters, Milford, MA), amino acid analysis of acid (HCI) hydrolysates and fast atom bombardment mass spectrophotometry.

The synthesis of the cyclic peptides included in Formula II may be achieved, for example by a solid-phase procedure using Kaiser's oxime-resin and following procedures of Osapay et al., (Tetrahedron Letters, 31, 6121-6124, 1990) and Nishino et al., (J. Chem. Soc. Kin Trans. 1, 939-946, 1986).

Another object of the invention is to provide pseudopeptides based on the structures of peptides of general Formulae I and II, wherein (CO-NH) bonds between amino acids

are replaced each independently by (CS-NH) or (CH_2-NH) bonds known as pseudopeptide bonds, said pseudopeptides possessing one or two pseudopeptide bonds of the same or different types for Formula I, and one, two or three pseudopeptide bonds of the same or different types for Formula II. Pseudopeptides may be obtained by a solid-phase procedure (Le, Michelot, Dumont, Shukla, Mayer, and Lemaire (Can. J. Physiol. Pharmacol., 75: 9-14, 1997), for example, according to the method of Michelot et al., (In "Innovation and perspectives in solid-phase synthesis, biological and biomedical applications Edited by R. Epton. Mayflower Worldwide Inc., Birmingham, in press.).

Another object of the invention is to provide retro-verso forms of tetrapeptides of general Formulae I and II, such peptides comprising, for example, Arg-Gly-Gln-Gly, Arg-Ala-Gln-Gly, Arg-Ala-Gln-Arg, cyclo(-Arg-Ala-Gln-Gly-) and cyclo(-Arg-Ala-Gln-Arg-) for the retro-verso forms of SL-99, SL-100, S-101, SL-102 and SL-103, respectively. The synthesis of these peptides can be as described above for peptides of Formulae I and II.

Another object of the invention is to provide a mechanism for a tetrapeptide to produce analgesia, said mechanism consists in blocking the activity of the central excitatory amino acid NMDA receptor.

Another object of the invention is to provide a method which consists in administering, centrally or peripherally, a peptide, said peptide HN fragments or analogues of HN

fragments represented in Formula I and Formula II, to treat pain.

A further aspect of the invention is a pharmaceutical composition comprising a peptide of general formula I or II, or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable diluent or carrier. The preparation and administration of pharmaceutical compositions may be by known methods, such as those described in U.S. Patent No. 5,169,833, which is herein incorporated by reference.

In yet another aspect the invention provides a commercial package, containing a peptide of general formula I or II, with instructions for its use in the treatment of pain.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is illustrated by the following non-limiting examples, which can be better understood with the aid of the figures.

Figure 1 shows a dose-response curve of the analgesic effects of HN, closed square, SL-100, open square, SL-101 open circle and SL-102 closed circle.

Figure 2 shows time response curves for the analgesic effects of [Ser^1]HN, closed square, 50 nmol/mouse, SL-100, closed triangle, 10 nmol/mouse and cyclo-(SL-100 or SL-102) open triangle 50 nmol/mouse.

Figure 3 shows the effect of naloxone (N), MK-801 (MK) and CPP on the analgesic effects of [Ser^1]HN (SHN, 50 nmol/mouse, i.c.v.).

Figure 4 shows the analgesic effect of peripheral administration of morphine, H4-(86-100), [Ser^1]HN, HN-(7-15), SL-101 and cyclic SL-100.

EXAMPLE 1

CENTRAL AND PERIPHERAL NON-OPIOID ANTINOCICEPTIVE EFFECTS OF GLY-GLN-ALA-ARG, ARG-GLN-ALA-ARG, CYCLO(-GLY-GLN-ALA-ARG-) AND RELATED PEPTIDES IN THE MOUSE WRITHING PAIN ASSAY: COMPARISONS WITH HISTOGRANIN AND MORPHINE.

MATERIALS AND METHODS

Animals

Mice (male 20-25 g, Swiss Webster) were obtained from Charles River (Canadian Breeding Farm, St. Constant, Quebec). They were housed five per cage in a room with controlled temperature (22 ± 2°C), humidity and artificial light (06.30-19h). The animals had free access to food and water and were used after a minimum of 4 days of acclimation to housing conditions. Experiments were carried out between 10:00 a.m. and 4:00 p.m. in an air-regulated and soundproof laboratory (23 ± 1°C, 40 % humidity), in which mice were habituated at least 30 min before each experiment. The experiments were authorized by the animal care committee of the University of Ottawa in accordance with the guidelines of the Canadian Council on Animal Care.

Drugs and peptides

(±)3-(2-carboxypiperazine-4-yl)-propyl-1-propionic acid (CPP) and (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d] cyclo-hepten-5,10-imine maleate (MK-801) were obtained from Tocris Neuramin, Essex, England. HN and related peptide analogues and fragments were synthesized in our laboratory by the solid-phase procedure (26) as described previously (27). The purity of the synthetic peptides was assessed by analytical HPLC on

μ-Bondapak C18 (Waters) and by thin-layer chromatography on silica gel plates (60 F 254; BDH Chemicals, Darmstadt, Germany) in the following solvent system (v/v) : 1-butanol /acetic acid / water / pyridine (15/3/10/12). Their composition and molecular weight were determined by amino acid analysis of acid (HCl) hydrolysates and fast atom bombardment mass spectrophotometry (FAB MS), respectively.

For the synthesis of cyclo(-Gly-Gln-Ala-Arg-) (SL-102), the Kaiser's oxime-resin was used following the procedures of Osapay et al (35) and Nishino et al (36). The starting compound, Boc-Ala-Oxim-Resin, was prepared from Oxim-Resin (Novabiochem, 1g, 0.47 meq/g) by using Boc-Ala-OH in the presence of PyBOP (3 eq), HOBt(1 eq), in DMF for 2h (repeated 2 times), and the excess oxim groups were capped by acetylation. The peptide chain was then assembled according to the following coupling steps: (i) two washes with DCM, (ii) one wash with 25% TFA-DCM, (iii) deprotection with 25% TFA-DCM (30 min), (iv) two washes with DCM, (v) one wash with propanol-2, (vi) three washes with DCM, (vii) one wash with DMF, (viii) coupling of Boc-amino-acids (consecutively Boc-Gln, Boc-Gly and Boc-Arg(Tos)(3 eq, each)) in presence of PyBOP (3 eq), HOBt(1 eq) and DIEA (5 eq) in DMF (45 min), (ix) three washes with DMF, (x) two washes with DCM. Solvent volumes were 15 $cm^3 g^{-1}$ resin. Coupling efficiency was checked by the Kaiser test (34). The free amino group cleaved the peptide from the polymer support by intrachain aminolysis in the presence of AcOH (2 eq) and DIEA (2 eq) in DMF at room temperature. After 24 h reaction time, the product was

obtained from the solution phase by filtration. Protecting group (Tos) of the peptide was removed with anhydrous HF at 0° C for 30 min. This crude product was purified by RP-HPLC (Bondapak C18 column, 10um x 125A, 25 x 100 mm, with the gradient of 30%-40% acetonitrile-ammonium acetate 5mM over 50 min) with final yield 15%, based on starting resin. The purity and identity of the synthetic peptide was assessed by analytical HPLC on Bondapak C18 column, 10um x 125A, 3.9 x 300 mm, with the gradient of 30%-40% acetonitrile-ammonium acetate over 50 min, t_R: 35 min, molecular mass by FAB-MS: 412 (calc.: 412.5, Dr. J. Wang, Mass Spectrometry Lab, Medical Sciences Bldg., Toronto, Canada), amino acid analysis: Ala(1)0.9, Arg(1)1.1, Gln(1)0.8, Gly(1)1 (Dr. R. Interior, The Biotechnology Service Centre, Department of Clinical Biochemistry, Toronto, Canada).

The i.c.v. administrations of the peptides were performed as described by Shukla et al. (28). Peptides were dissolved in double-distilled sterile water (vehicle) and 10 µl of the peptide solution or vehicle were delivered gradually within approximately 3 sec, mice exhibiting normal behaviour within 1 min after injection. The administration site was confirmed by injecting Indian ink in preliminary experiments.

Antinociceptive assay

Antinociceptive activity of HN and related compounds was evaluated using the acetic acid-induced writhing test according to a modification (28) of the method of Hayashi and Takemori (29). Male Swiss Webster [(SW)f BR] mice were injected intraperitoneally (i.p.) with 1.0% acetic acid

(10ml/kg) 5 min after i.c.v. injection of 0 (saline), 0.5, 1, 10 , 25, 50, 75 and 100 nmol of HN or related peptides. The number of writhes displayed by each mouse was counted for a period of 10 min after the injection of the acetic acid solution. An abdominal stretch is characterized by the contraction of the abdominal muscles, the arching of the back ventrally such as the abdomen touches the bedding surface and the extension of one or both hind limbs. Mice were used once and then killed immediately. Groups of 10 mice were used for each dose. The compound was said to be active at a given dose if after its administration, the number of writhes elicited by a mouse injected with acetic acid was equal to, or less than, one-half the median number of writhes recorded for the saline-treated control group of mice that day, as described by R.I. Taber (37). The results are expressed in terms of either the number of mice out of ten in which a given dose of a peptide was considered to be active or the ED_{50} value (the dose of the peptide that produced analgesia in 50% of the animals). The ED_{50} values with 95% confidence limits (95% CL) and potency ratios with 95% CL were measured by the method of Lichfield and Wilcoxon (30) using procedure 47 of the computer program of Tallarida and Murray (31). In order to determine the length of action of [Ser^1]HN and related peptides, the acetic acid solution was administered at different times after the administration of the peptide, as indicated. For verifying the blockade of the analgesic effect of the peptides with receptor antagonists, naloxone (1nmol), MK-801 (0.3 nmol) or CPP (0.1 nmol) were administered i.c.v. in an aliquot of 10

μl, alone or in combination with [Ser^1]HN (50 nmol) or related peptides. The experiments for assessment of the peripheral antinociceptive activity of the peptides were performed by i.p. administration of 5 μmol/kg of the tested compounds 10 min prior to the injection of the acetic acid solution. Data were analyzed by the Wilcoxon's paired non-parametric test. The criterion for statistical significance was $P < 0.05$.

RESULTS

Antinociceptive efficacy of histogranin and related peptides

Intracerebroventricular administration of HN and related peptides in mice induced dose- and structure-dependent analgesic activities as assessed by their ability to inhibit writhing in response to acetic acid (i.p.; fig. 1). Histone H4-(86-100) was 5.4 times more potent than HN with an ED_{50} of 4.1 nmol/mouse as compared with 22.3 nmol/mouse for HN (table 1). The chemically stable HN analogue, [Ser^1]HN, displayed an analgesic potency similar to that of HN. The unmodified C-terminal fragment of histone H4, osteogenic growth peptide (OGP; 32), was 2 times less potent than HN. The analgesic activity of HN was shown to reside in the C-terminal portion of the peptide, since HN-(7-15) was 3.0 times more potent than HN itself with an ED_{50} of 7.5 nmol/mouse, while the N-terminal fragment, HN-(1-10), was inactive at 50 nmol/mouse. The minimal core peptide for analgesic activity was HN-(7-10) (SL-99), with a potency ratio of 2 as compared with HN. [Ala^9]HN-(7-10) (SL-100) was 5.7 times as potent as HN, whereas the cyclic form of this peptide (SL-102) was 7.7 times as potent as HN (ED_{50} of 2.9 nmol/mouse; fig.1). [Arg^7, Ala^9]HN-(7-10)

(SL-101 or [Ala^{95}]H4-(92-95)) was 4.5 times as potent as HN, whereas the pentadecapeptide [Ser^{1}, Ala^{9}]HN (SL-104) was 5.4 times as potent as HN (table 1).

[Ser^{1}]HN (50 nmol/mouse) produced an analgesic effect that lasted approximately 45 min (fig. 2). Its maximal antinociceptive effect lasted approximately 15 min. The tetrapeptide SL-100 (10 nmol/mouse) produced an effect that lasted only 15 min. However, cyclization of this tetrapeptide (SL-102) greatly enhanced its length of action (an effect that lasted more than 45 min after i.c.v. injection of the compound, Fig 2).

NMDA receptor-mediated analgesic activity

In order to verify which receptor was involved in the antinociceptive activity of [Ser^{1}]HN, the peptide was coadministered i.c.v. with the opioid antagonist naloxone or the competitive or non-competitive NMDA antagonists, CPP and MK-801, respectively (fig. 3). Naloxone alone (1 nmol/mouse) did not show significant analgesic activity in the mouse writhing test, and in combination with [Ser^{1}]HN (50nmol/mouse), it did not significantly modify the antinociceptive activity of the peptide (fig. 3). Both NMDA receptor antagonists, CPP (0.1 nmol/mouse) and MK-801 (0.3 nmol/mouse) injected alone, did not display significant analgesic activity; but they significantly antagonized the analgesic activity of [Ser^{1}]HN (50 nmol/mouse; fig. 3).

Peripheral Activity

Peripheral (i.p., i.v. and s.c.) administration of dynorphin A-(1-13) was recently shown to cause analgesia in

the mouse writhing assay (33). In order to verify if the naloxone-insensitive antinociceptive effects of HN related peptides could be observed after such type of administration, histone H4-(86-100), [Ser^1]HN, HN-(7- 15), SL-100 and cyclic SL-100 (or SL-102) were administered i.p. at 5 μmol/kg, and the percentage of mice showing analgesia was measured 10 min after the injection of the peptides and compared with that obtained with morphine (5 μmol/kg, i.p.; fig. 4). The five peptides produced significant antinociceptive activity, the incidence of positive responses being 50%, 63%, 62% and 65%, respectively, as compared with 90% for morphine.

Table 1: Relative potency of histogranin (HN) and related peptides (i.c.v.) in producing analgesia in the mouse writhing pain assay.

Peptide		ED_{50} (nmol/mouse) (95% CL)[a]		Potency ratio[b] (95% CL)	
HN		22.3	(12.1-41.1)	1.0	
H4-(86-100)		4.1	(0.9-17.9)	5.4	(0.7-40.1)
[Ser^1]HN		17.4	(7.0-43.0)	1.3	(0.4-3.8)
H4-(89-102) (OGP)[c]		40.9	(25.8-65)	0.5	(0.25-1.17)
HN-(7-15)		7.5	(2.3-24.4)	3.0	(0.8-11.2)
HN-(1-10)		NA			
HN-(7-10)	**(SL-99)**	**11.3**	**(4.2-30.4)**	**2.0**	**(0.6-6.3)**
[Ala^9]HN-(7-10)	**(SL-100)**	**3.9**	**(1.7-9.1)**	**5.7**	**(2.0-15.9)***
[Arg^7, Ala^9]HN-(7-10)	(SL-101)	4.9	(1.8-13.2)	4.5	(1.4-14.3)*
cyclo-(SL-101)	(SL-102)	2.9	(0.8-9.8)	7.7	(1.3-46.6)*
[Ser^1, Ala^9]HN	**(SL-104)**	**4.1**	**(1.5-11.5)**	**5.4**	**(1.6-18.0)***

[a]95% Confidence limit. [b]As compared with HN. [c]OGP: osteoblastic growth peptide. NA: not active at 50 nmol/mouse (i.c.v.). * $P \leq 0.05$ as compared with HN.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A peptide designated histogranin-(7-10) or "HN-(7-10)" having the structure:

H-Gly-Gln-Gly-Arg-COOH [SL-99] .

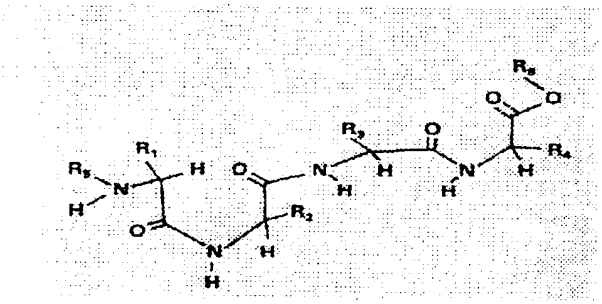
2. A peptide designated [Ala^9]HN-(7-10) having the structure of:

H-Gly-Gln-Ala-Arg-COOH [SL-100] .

3. A peptide designated [Arg^7, Ala^9]HN-(7-10) having the structure of:

H-Arg-Gln-Ala-Arg-COOH [SL-101] .

4. A series of peptides according to claim 2, said peptides with general Formula I:



and the pharmaceutically-acceptable salts, esters and amides, wherein:

R_1 is hydrogen or an alkyl radical (the term alkyl as used herein means a hydrocarbon radical having from one to ten carbon atoms, which can be a straight or branched chain, and including from zero to four carbon-carbon double or triple bonds. Representative of such radicals are methyl, ethyl, n-propyl, isopropyl, n-butyl, 2-ethyl-hexyl and the like.);

R_2 is an amide radical such as $(CH_2)_n$-$CONH_2$, "n" an integer from 0 to 10;

R_3 is hydrogen or an alkyl radical as defined above;

R_4 is a basic radical (the term basic as used herein means $(CH_2)_n$-NH_2 or $(CH_2)_n$-NH-C(=NH)NH_2, "n" each independently an integer from 0 to 10);

R_5 is hydrogen or an acetyl or an alkyl radical as defined above;

R_6 is hydrogen, alkyl, alkyl carbonyl, alkoxy carbonyl, amino carbonyl, alkylaminocarbonyl, dialkylamino, carbonyl, $(CH_2)_n$-benzyl, $(CH_2)_n$-phenyl, ("n" an integer from 1 to 10);

O-R_6 is replaced by R_7 (not shown), R_7 being amino, hydroxy, alkoxy, alkylamino, dialkylamino, or alkoxyaryl;

O-R_6 is replaced by R_7, R_7 being independently positions 11 to 15 in HN and represented by Thr^{11}-Leu-Tyr-Gly-Phe^{15}, Thr^{11}-Leu-Tyr-Gly^{14}, Thr^{11}-Leu-Tyr^{13}, Thr^{11}-Leu^{12} and Thr^{11}.

5. A series of peptides according to claim 3, said peptides with general Formula I (shown in claim 4), and the pharmaceutically-acceptable salts, esters and amides, wherein:

R_1 is a basic radical. The term basic as used herein means $(CH_2)_n$-NH_2 or $(CH_2)_n$-NH-C(=NH)NH_2, "n" each independently an integer from 0 to 10);

R_2 is an amide radical such as $(CH_2)_n$-$CONH_2$, "n" an integer from 0 to 10;

R_3 is hydrogen or an alkyl radical as defined above;

R_4 is a basic radical as defined above;

R_5 is hydrogen or an acetyl or an alkyl radical as defined above;

R_6 is hydrogen, alkyl, alkyl carbonyl, alkoxy carbonyl, amino carbonyl, alkylaminocarbonyl, dialkylamino, carbonyl, $(CH_2)_n$-benzyl, $(CH_2)_n$-phenyl, ("n" an integer from 1 to 10).

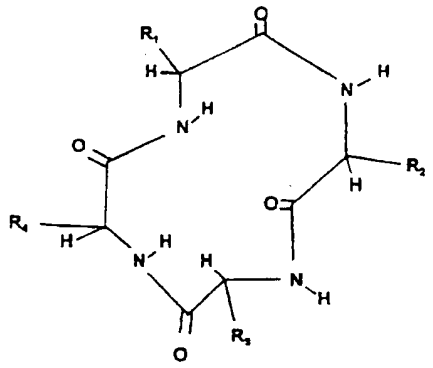
O-R_6 is replaced by R_7 (not shown), R_7 being amino, hydroxy, alkoxy, alkylamino, dialkylamino, or alkoxyaryl.

O-R_6 is replaced by R_7, R_7 being independently positions 11 to 15 in HN and represented by Thr^{11}-Leu-Tyr-Gly-Phe^{15}, Thr^{11}-Leu-Tyr-Gly^{14}, Thr^{11}-Leu-Tyr^{13}, Thr^{11}-Leu^{12} and Thr^{11}.

6. A cyclic peptide according to claim 2 designated cyclo[Ala^9]HN-(7-10) with the following structure:

cyclo(-Gly-Gln-Ala-Arg-) [SL-102] .

7. A series of peptides according to claim 6, said peptides with general Formula II:



wherein:

R_1 is hydrogen or an alkyl radical (the term alkyl as used herein means a hydrocarbon radical having from one to ten carbon atoms, which can be a straight or branched chain, and including from zero to four carbon-carbon double or triple bonds. Representative of such radicals are methyl, ethyl, n-propyl, isopropyl, n-butyl, 2-ethyl-hexyl and the like.);

R_2 is an amide radical such as $(CH_2)_n$-$CONH_2$, "n" an integer from 0 to 10;

R_3 is hydrogen or an alkyl radical as defined above;

R_4 is a basic radical (the term basic as used herein means $(CH_2)_n$-NH_2 or $(CH_2)_n$-NH-C(=NH)NH_2, "n" each independently an integer from 0 to 10).

8. A cyclic peptide according to claim 3 designated cyclo[Arg ^{7}, Ala^9]HN-(7-10) with the following structure:

cyclo(-Arg-Gln-Ala-Arg-) [SL-103] .

9. A series of peptides according to claim 8, said peptides with general Formula II (shown in claim 7), wherein:

R_1 is a basic radical. The term basic as used herein means $(CH_2)_n$-NH_2 or $(CH_2)_n$-NH-C(=NH)NH_2, "n" each independently an integer from 0 to 10);

R_2 is an amide radical such as $(CH_2)_n$-$CONH_2$, "n" an integer from 0 to 10;

R_3 is hydrogen or an alkyl radical as defined above;

R_4 is a basic radical as defined above.

10. A series of pseudopeptides, based on the structures of peptides of general Formulae I of claim 4 and II of claim 7, wherein pseudopeptide bonds comprising (CS-NH) or (CH_2-NH) bonds are introduced between amino acids each independently, said pseudopeptides possessing one or two pseudopeptide bonds of the same or different types for peptides of Formula I, and one, two or three pseudopeptide bonds of the same or different types for peptides of Formula II.

11. A series of retro-verso forms of the tetrapeptides of general Formulae 1 of claim 4 and II of claim 7, such peptides comprising Arg-Gly-Gln-Gly, Arg-Ala-Gln-Gly, Arg-Ala-Gln-Arg, cyclo(-Arg-Ala-Gln-Gly-) and cyclo(-Arg-Ala-Gln-Arg-) for the retro-verso forms of SL-99, SL-100, SL-101, SL-102 and SL-103, respectively.

12. A mechanism for a tetrapeptide to produce analgesia, said mechanism consists in blocking the activity of the central excitatory amino acid NMDA receptor.

13. A method of treating pain, said method comprising administering to said mammal an effective dose of a peptide, said peptide being SL-99, SL-100, SL-101, SL-102, SL-103, SL-104 and all other peptides comprised in Formula I and Formula II or pseudopeptides derived from these compounds.

14. Use of a peptide or pseudopeptide, as claimed in any one of claims 1 to 11 for the treatment of pain.

15. A use according to claim 14, wherein the pain is chronic pain.

16. Use of a peptide or pseudopeptide, as claimed in any one of claims 1 to 11 for the preparation of a pharmaceutical composition for the treatment of pain.

17. A pharmaceutical composition for the treatment of pain comprising a peptide or pseudopeptide as claimed in any one of claims 1 to 11, or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable diluent or carrier.

18. A commercial package containing a peptide or pseudopeptide as claimed in any one of claims 1 to 11, or a pharmaceutically acceptable salt thereof, and instructions for its use in the treatment of pain.

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PATENT AGENTS

Figure 1

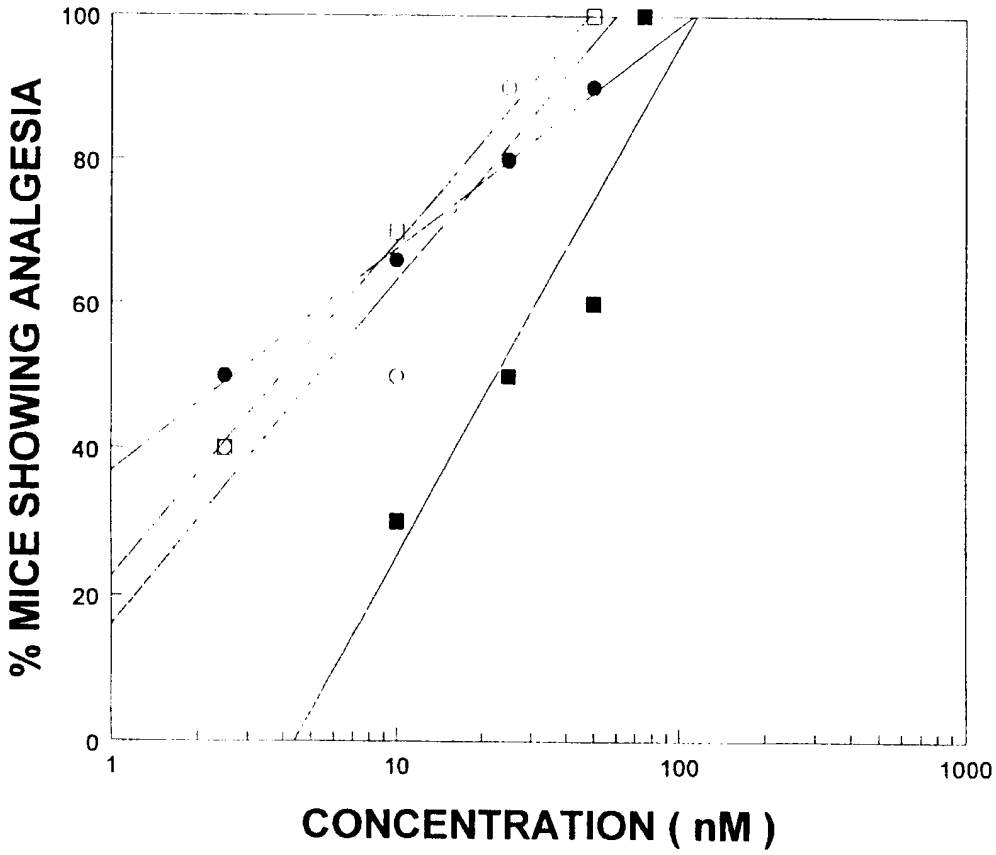

% MICE SHOWING ANALGESIA
100
80
60
40
20
0
1
10
100
1000
CONCENTRATION (nM)

Figure 2

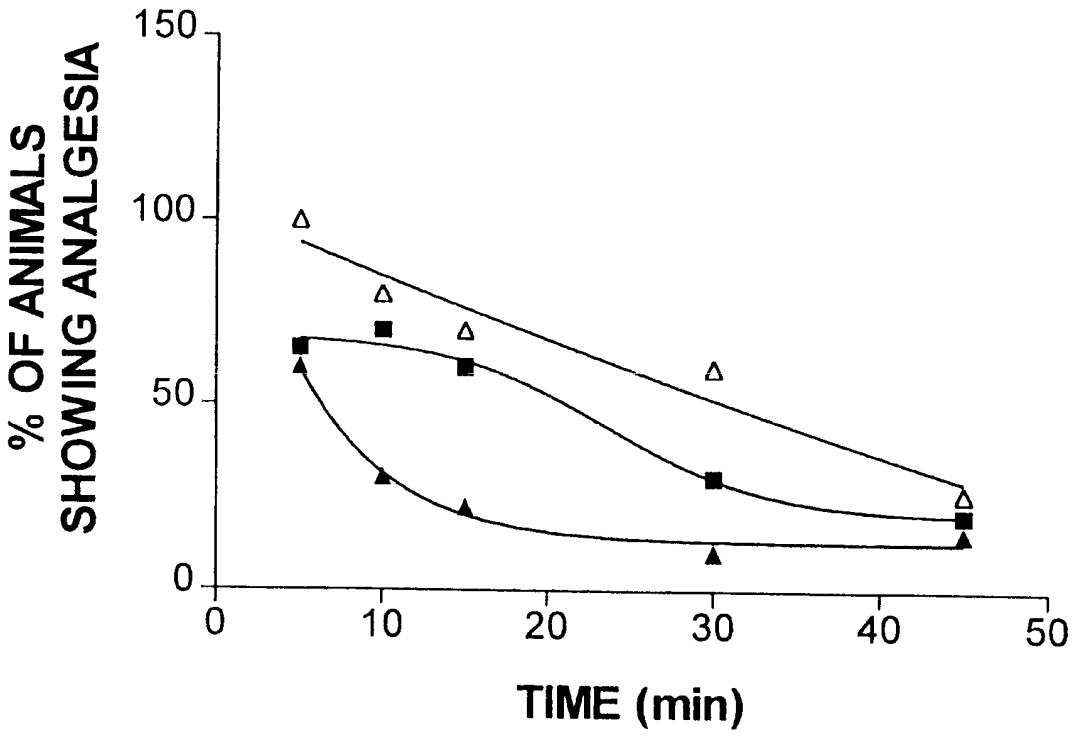

% OF ANIMALS SHOWING ANALGESIA
150
100
50
0
0
10
20
30
40
50
TIME (min)

Figure 3

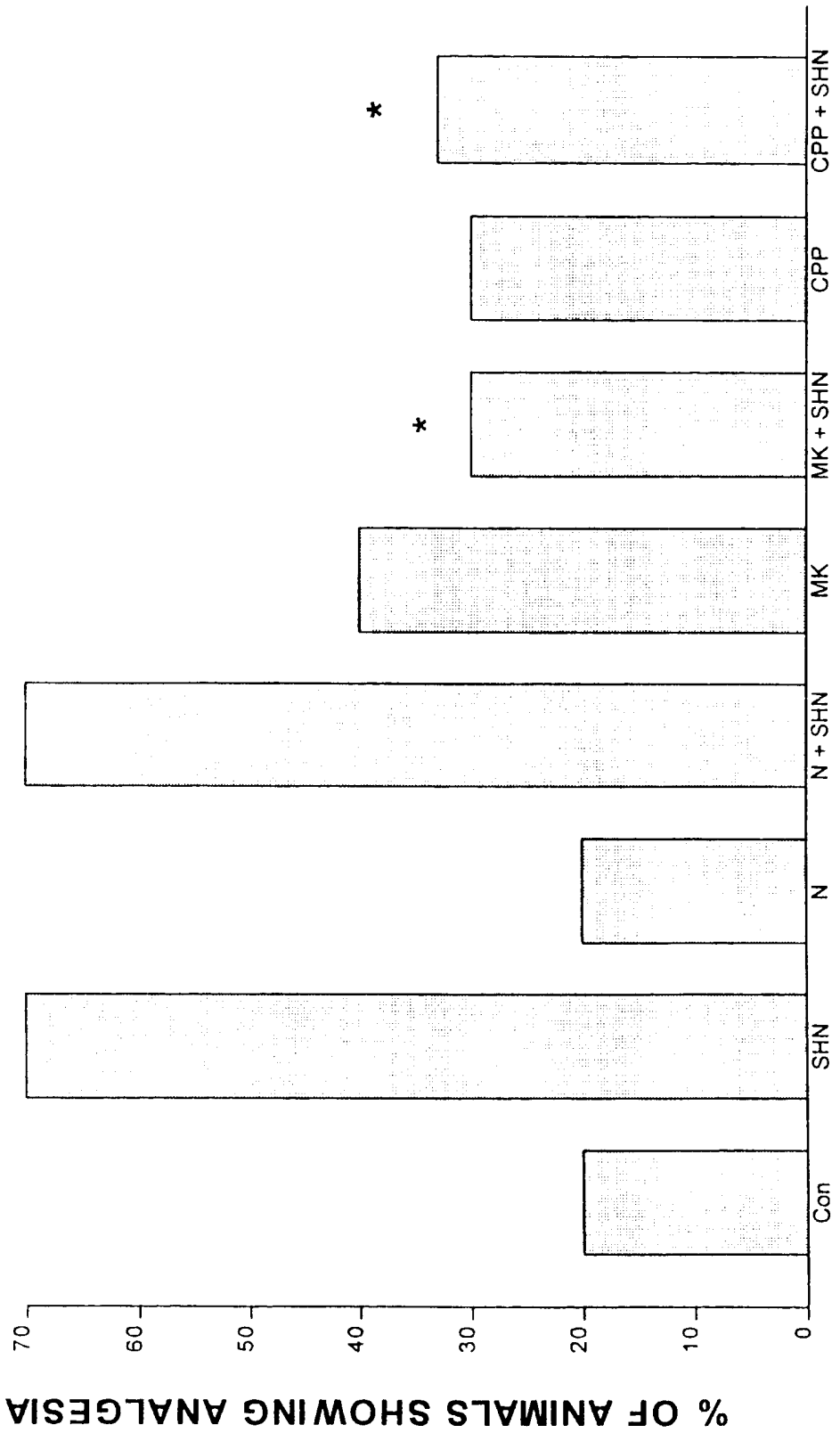

% OF ANIMALS SHOWING ANALGESIA
0
10
20
30
40
50
60
70
Con
SHN
N
N + SHN
MK
MK + SHN
CPP
CPP + SHN
*
*

Figure 4

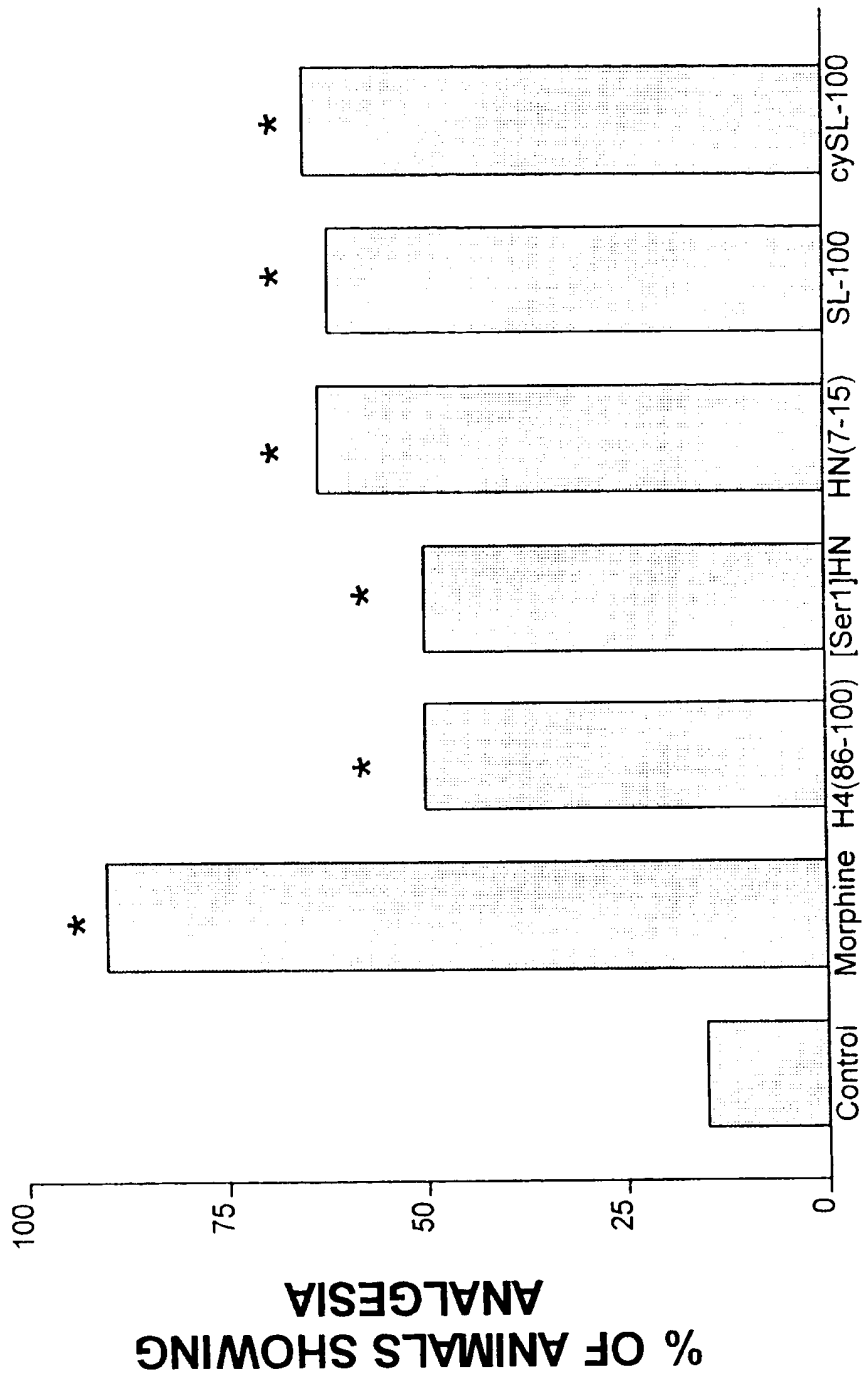

% OF ANIMALS SHOWING ANALGESIA
100
75
50
25
0
Control
Morphine
H4(86-100)
[Ser1]HN
HN(7-15)
SL-100
cySL-100
*
*
*
*
*
*

Figure 1

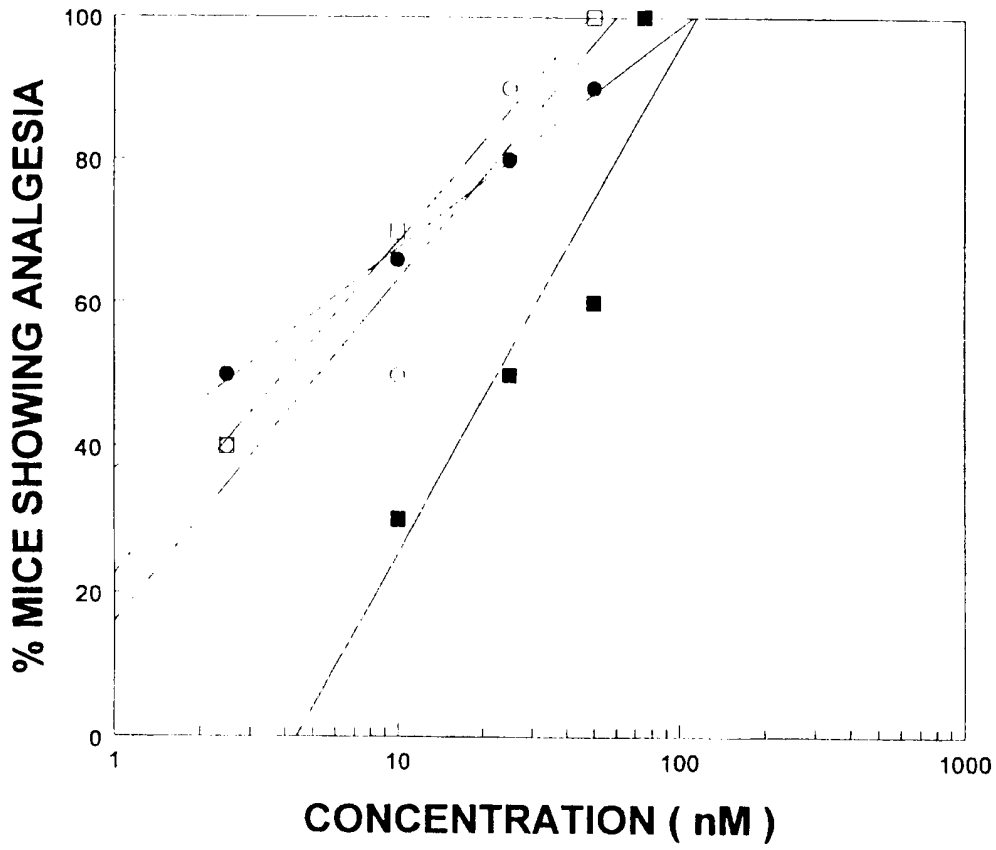

% MICE SHOWING ANALGESIA
100
80
60
40
20
0
1
10
100
1000
CONCENTRATION (nM)

Figure 2

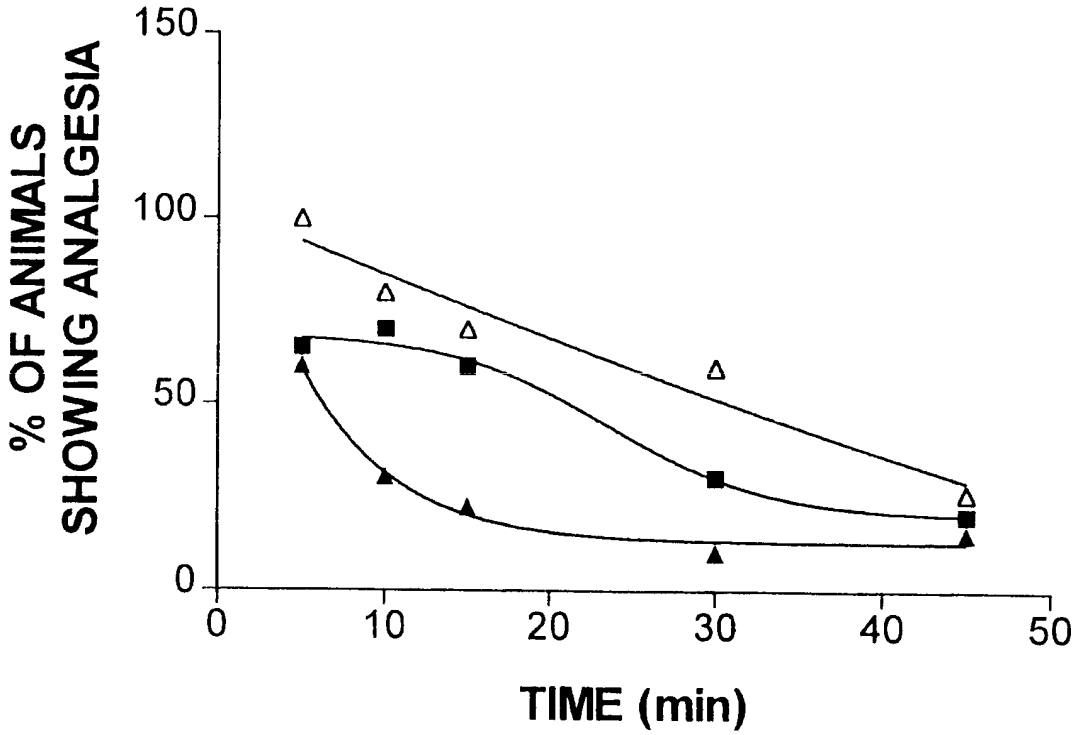


Figure 3

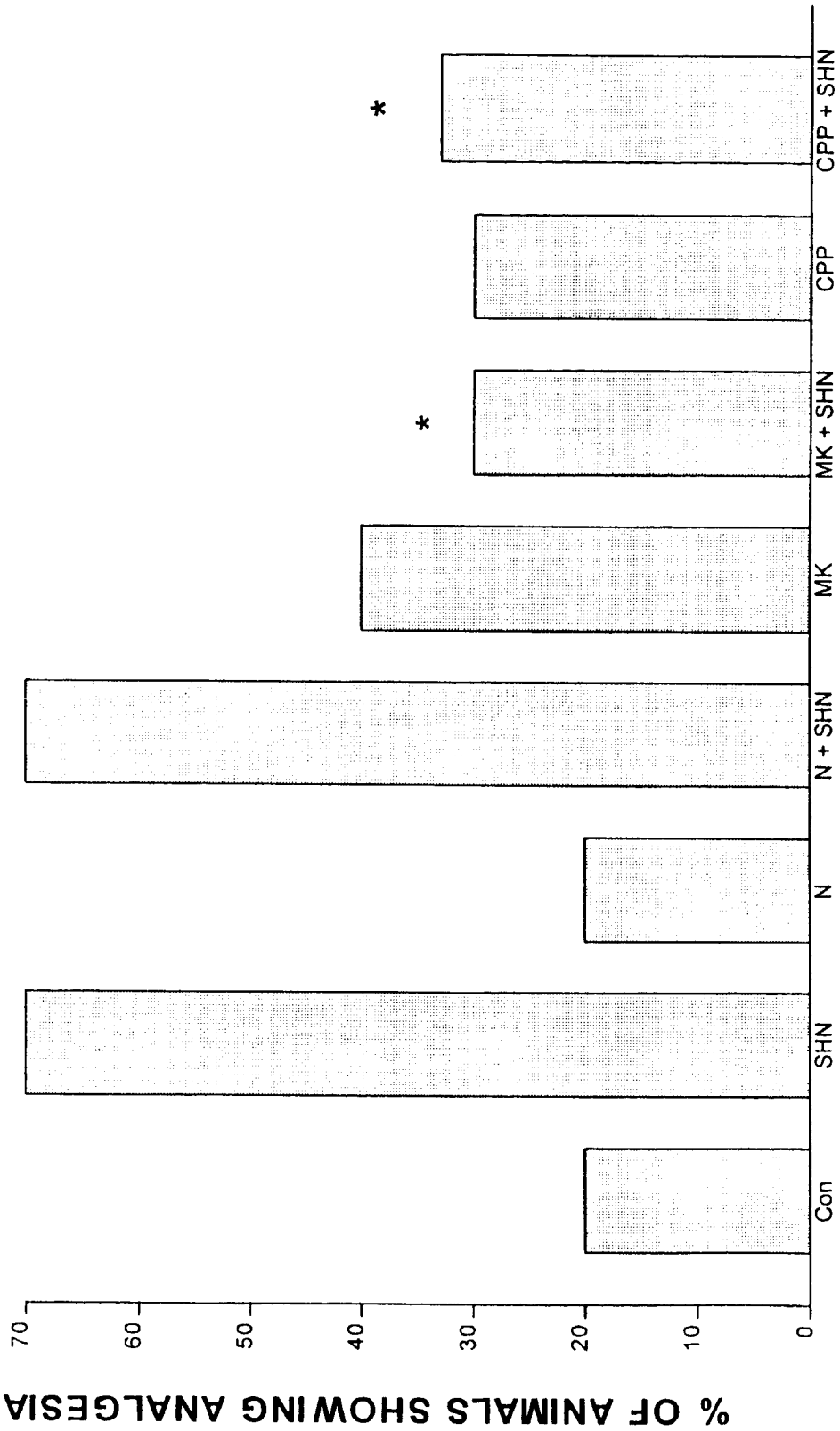
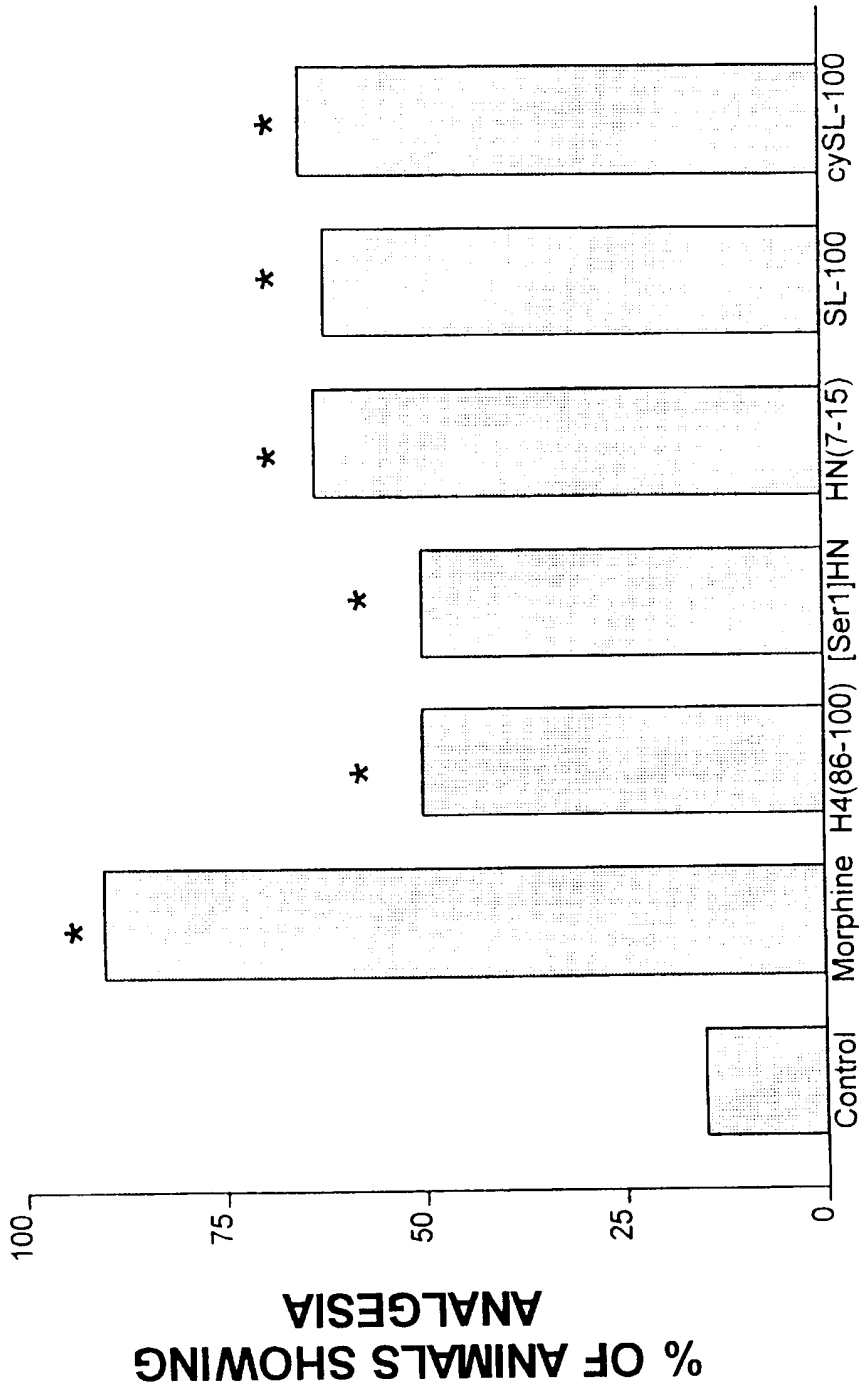

% OF ANIMALS SHOWING ANALGESIA
0
10
20
30
40
50
60
70
Con
SHN
N
N + SHN
MK
MK + SHN
CPP
CPP + SHN
*
*

Figure 4


% OF ANIMALS SHOWING ANALGESIA
100
75
50
25
0
Control
Morphine
H4(86-100)
[Ser1]HN
HN(7-15)
SL-100
cySL-100
*
*
*
*
*
*